FEBS Letters 587 (2013) 1703–1710

journal homepage: www.FEBSLetters.org

Review

The Evi5 family in cellular physiology and pathology

Yi Shan Lim [a], Bor Luen Tang [a,b,*]

[a] Department of Biochemistry, Yong Loo Lin School of Medicine, National University Health System, Singapore
[b] NUS Graduate School of Integrative Sciences and Engineering, National University of Singapore, 8 Medical Drive, Singapore 117597, Singapore

ARTICLE INFO

Article history:
Received 25 February 2013
Revised 23 April 2013
Accepted 28 April 2013
Available online 10 May 2013

Edited by Lukas Huber

Keywords:
Cell cycle
Evi5
GTPase Activating Protein (GAP)
Rab

ABSTRACT

The Ecotropic viral integration site 5 (Evi5) and Evi5-like (Evi5L) belong to a small subfamily of the Tre-2/Bub2/Cdc16 (TBC) domain-containing proteins with enigmatically divergent roles as modulators of cell cycle progression, cytokinesis, and cellular membrane traffic. First recognized as a potential oncogene and a cell cycle regulator, Evi5 acts as a GTPase Activating Protein (GAP) for Rab11 in cytokinesis. On the other hand, its homologue Evi5L has Rab-GAP activity towards Rab10 as well as Rab23, and has been implicated in primary cilia formation. Recent genetic susceptibility analysis points to Evi5 as an important factor in susceptibility to multiple sclerosis. We discuss below the myriad of cellular functions exhibited by the Evi5 family members, and their associations with disease conditions.

1. Introduction

The mouse gene locus Ecotropic viral integration site 5 (Evi5) was first identified as a common site for retroviral integration at mouse chromosome 5 in T-cell lymphomas of recombinant inbred AKXD mice with high incidences of lymphoma [1]. Pro-viral integrations at the *Evi5* locus disrupt a gene [2] – located 18 kb upstream of another common viral integration site, Growth factor independence 1 (*Gfi1*) (which itself encodes a zinc finger-containing transcription factor involved in interleukin-2 signaling). The human orthologue was separately identified as part of the constitutional, reciprocal, t(1;10)(p22q21) translocation breakpoint in a patient with stage 4S neuroblastoma. The corresponding gene on chromosome 1p22, also named NB4S [3], encodes a 7.5 kbp transcript with an 810 amino acid open reading frame expressed in a wide variety of tissues. A paralogue, known as Evi5 homologue [4] or Evi5-like [5], shares about 70% identity to Evi5 and contains the same domain structure, is located on mouse chromosome 8 and human chromosome 19 respectively.

Human and mouse Evi5 and Evi5-like encodes multiple alternatively spliced transcripts. The longest polypeptide encoded by these two proteins share two prominent domain features–a Tre-2/Bub2/Cdc16 (TBC) domain [6] at the N-terminal half, as well as a coiled-coil region that bears homology to Structural Maintenance of Chromosomes (SMC) family of ATPases [7] at the C-terminal half (Fig. 1). TBC domains are commonly associated with a functional class of proteins that act as GTPase Activating Proteins (GAPs) for the Rab family GTPases that regulate membrane traffic [8]. SMC proteins, on the other hand, are an evolutionarily conserved group of molecules that are important in maintaining genome integrity [7,9]. In eukaryotes, they are core components of the condensin and cohesin complexes responsible for regulating chromosomal condensation, pairing and segregation [10]. The physiological functions of Evi5 are only beginning to be unraveled, and characterization of Evi5's localization and activities indicate that it may serve different functions during the course of cell division by engaging different interacting partners. Furthermore, Evi5L may be functionally distinct from Evi5 in spite of their high degree of homology. We discuss in the paragraphs below, previously known and recently revealed cellular activities exhibited by the Evi5 family members, as well as their implicated or predicted involvements in disease pathology.

2. Evi5 as a modulator of events during cell division

The initial reports describing the discovery of Evi5 shed little light on its possible function other than hinting that the ubiquitously expressed gene product, or truncated forms of it, could contribute to oncogenesis, which is unsurprising given the link between TBC domains and cell cycle regulation [2,3]. A subsequent report indicated that Evi5 has a putative nuclear localizing signal and exhibited a diffused nucleoplasm staining [11]. Interestingly, Evi5 is a phosphoprotein and has a prominent centrosomal

* Corresponding author at: NUS Graduate School of Integrative Sciences and Engineering, National University of Singapore, 8 Medical Drive, Singapore 117597, Singapore. Fax: +65 6779 1453.
E-mail address: bchtbl@nus.edu.sg (B.L. Tang).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.04.036

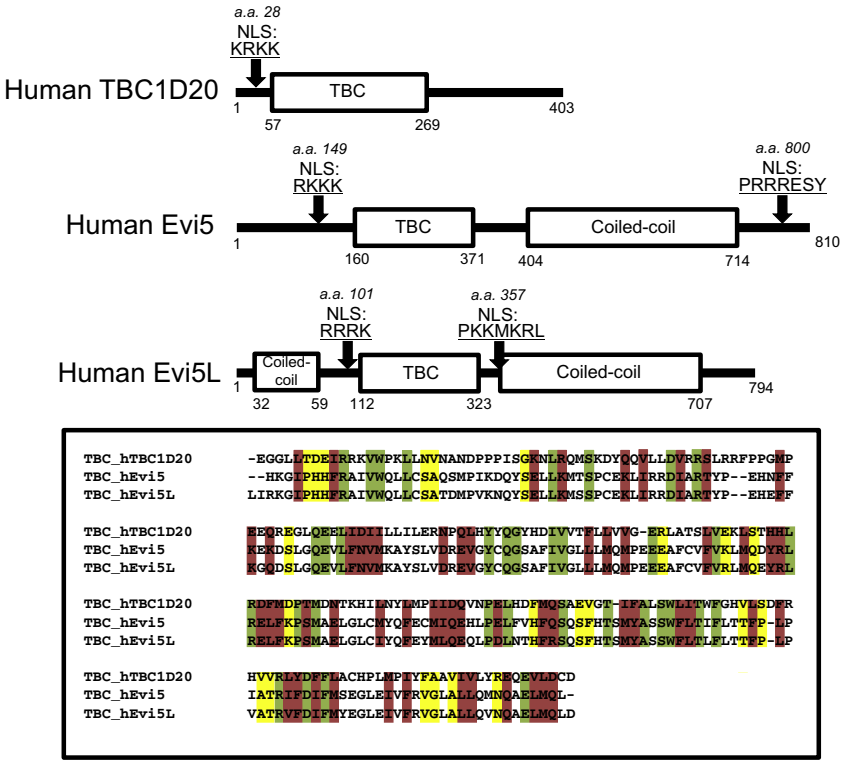

Fig. 1. Schematic diagram of human TBC1D20, Evi5 and Evi5L. Protein sequence analyses of complete sequences of human TBC1D20 (an example of a known RabGAP that accelerates hydrolysis of Rab1), human Evi5 and human Evi5L, were carried out using two public domain software, Interpro (http://www.ebi.ac.uk/interpro/) protein sequence analysis and classification, and PSORT II prediction (http://psort.hgc.jp/form2.html). Boxes and arrows mark out the relative positions of TBC domains and the coiled-coil domains. The numbers along the protein sequence depict the relative amino acid positions of where the respective regions begin. The multiple alignment analysis was performed by ClustalW (http://embnet.vital-it.ch/software/ClustalW.html). Green highlighting marks residues that are identical between the TBC domains of TBC1D20, Evi5 and Evi5L, pink highlights refer to conserved substitutions, while yellow highlights the semi-conserved substitutions.

localization in interphase cells. It also co-precipitates with both α- and γ-tubulin. This differential subcellular localization patterns indicate that Evi5 could potentially act in different compartments of the cell.

Further work revealed that Evi5 undergoes several post-translational modifications, and has a dynamic association with mitotic structures during the M phase [12]. It decorates the mitotic spindle through anaphase and remains within the mid-zone and mid-body until the completion of cytokinesis. The protein is phosphorylated in early mitosis and undergoes proteolytic cleavage during late mitosis and cytokinesis. Silencing of Evi5 resulted in a multinucleate phenotype, suggesting a role for Evi5 in the completion of cytokinesis. In this regard, it is intriguing that Evi5 was shown to associate with the Chromosomal Passenger Complex (CPC) consisting of Aurora B kinase, Inner Centromere Proteins (INCENP), survivin and borealin [12]. The CPC acts in multiple processes during mitosis that safeguards genomic integrity during cell division. During cytokinesis, while daughter cells are still connected by chromosome bridges, the CPC limits chromosomal damage by imposing an abscission delay [13]. A recent report suggests that the CPC controls abscission timing through Aurora B kinase mediated-phosphorylation and inhibition of the vacuolar-sorting protein Snf7 component of the Endosomal Sorting Complex Required for Transport-III (ESCRT-III) polymerization and membrane association [14]. The exact role of Evi5 in cytokinesis, and whether it acts in this regard via its association with the CPC, is still unknown. On the other hand, another hypothesis proposed is that Evi5 exerts its effect in cytokinesis via its control of Rab11 activity [15,16]. This shall be discussed in more detail below.

Evi5 has also another implicated role in the earlier phases of the cell cycle – as a regulator of cyclin accumulation via its effects on the stability of Early mitotic inhibitor 1 (Emi1) accumulation [4]. Emi1 is an inhibitor of anaphase promoting complex/cyclosome (APC/C) complex, and together with either of its activators Cdc20 or Cdh1, sustain levels of APC/C's cyclin substrates [17,18]. Even in the absence of transcription factor E2F activation and basal levels of cyclin A transcription, Emi1's overexpression is apparently sufficient to drive S phase by stabilizing cyclin A [18]. Before mitosis, at the G1/S phase and throughout the S phase, Evi5 binds to a site adjacent to Emi1's degron, blocking degron phosphorylation by Polo-like kinases (Plks) [4], particularly Plk1 [19], thereby allowing the accumulation of Cyclin A for the progression of S phase [20]. Upon the onset of mitosis, phosphorylation of Emi1 leads to degradation by the Skp, Cullin, F-box (Fb) containing protein (SCF) E3 ubiquitin ligase complex, one of which consists of a β-transducin repeat containing protein (βTrCP)/Fbw1a [21]. Finally, phosphorylated Evi5 and Emi1 are then destined for ubiquitination and degradation by the 26S proteasome. Hence, Evi5 effectively maintains Emi1 levels in the S/G2 phase. Evi5 silencing induces aberrant and untimely degradation of Emi1, resulting in a sequence of events ranging from premature APC/C activation and cyclin destruction, culminating in cell-cycle arrest and mitotic catastrophe. Taken together, by acting through different interacting partners, Evi5 is able to exert varying influences on cell cycle progression (Fig. 2).

3. Evi5 as a Rab11 GAP

The Rab family of small GTPases regulate a myriad of membrane transport processes in the eukaryotic cell [22,23] via their engagement of effector molecules such as motors, tethering factors, coat proteins and membrane fusion factors [24–27] in their active GTP-bound form. Two classes of proteins principally

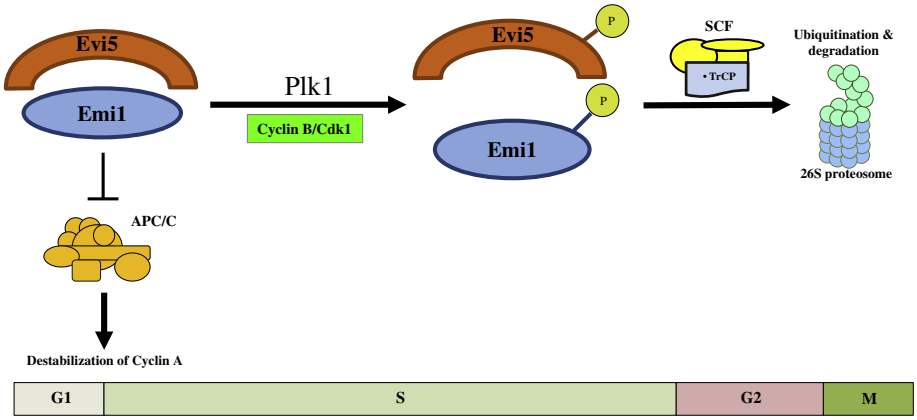

Fig. 2. Evi5's role in early stages of the cell cycle. Evi5 is a regulator of cyclin accumulation via its effects on the stability of the Early mitotic inhibitor 1 (Emi1) [4], the latter being an inhibitor of anaphase promoting complex/cyclosome (APC/C) complex. Before mitosis, at the G1/S phase and throughout the S phase, Evi5 binds to a site adjacent to Emi1's degron, blocking its phosphorylation by Polo-like kinase 1 (Plk1), thereby allowing the accumulation of Cyclin A for the progression into S phase. Cyclin B/Cdk1 appears to "prime" or enhance the phosphorylation of Emi1 by Plk1 [19]. Plk1 is also able to stimulate the phosphorylation of Evi5. Upon the onset of mitosis, Emi1 levels are regulated via degradation by the Skp, Cullin, F-box (Fb) containing protein (SCF) and β-transducin repeat containing protein (βTrCP) E3 ubiquitin ligase complex [21]. Phosphorylated Evi5 and Emi1 are then destined for ubiquitination and degradation by the 26S proteasome. See text for relevant references and sources.

regulate the GTP-binding status of Rabs, namely the Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins [28]. GEFs catalyze the GTP–GDP exchange of the Ras superfamily of small GTPases to effectively activate them [29]. A small number of Rab GEFs since identified have been found to be largely structurally unrelated to each other. However, certain Rab GEFs do form small functional subfamilies that are distinguishable by signature domains such as Vacuolar protein sorting 9 (Vps9) [30] and differentially expressed in normal and neoplastic cells (DENN) [31] domains.

The intrinsic GTP hydrolysis activity of Rab proteins is usually very low, and requires enzymatic activity induced by their respective Rab GAP proteins. GAPs are important in enhancing hydrolysis of the GTP-bound Rab, thereby switching activated Rabs to the inactive state [32]. These proteins therefore serve to restrict spatial and temporal overlap of Rabs, by mediating sequential steps of membrane transport [33]. Compared to GEFs, known Rab GAPs are somewhat less diverse, with Tre-2/Bub2/Cdc16 (TBC) domain-containing proteins expected to function as a Rab GAP [8,28]. The TBC domain was first recognized as a domain that was conserved among the *tre-2* oncogene product and yeast cell cycle regulators Bub2 and Cdc16, beginning with the cloning of mammalian TBC1 [34], has to date been discovered in more than 40 other proteins in humans and mice [8]. The GAP of Ypt6p (Gyp6p), the first Rab GAP, was cloned from yeast [35]. Several others, including those from mammals acting principally on Rab5 and Rab6, were subsequently identified [36–40]. A yeast 2-hybrid-based screen and subsequent in vitro Rab GAP activity assays identified seven TBC domain-containing proteins that interact with specific Rabs, of which a fraction of these proteins exhibit significant Rab GAP activity [5]. Although the TBC domain is proposed to be important for Rab interaction and best known as the RabGAP domain, a recent protein interaction screen for Rab effectors (i.e. which binds to the activated form of Rabs) identified three known GAPs, namely, mKIAA1055/TBC1D2B (Rab22-binding protein), GAPCenA/TBC1D11 (Rab36-binding protein) and centaurin β2 (Rab35-binding protein, which does not contain a TBC domain). Notably, all these three GAP proteins appear to interact with their specific Rabs via a domain other than the TBC domain [41].

Incidentally, two reports that were published in 2007 both demonstrated Evi5's interaction with members of the Rab11 family, also drew differing conclusions with regards to Evi5's GAP activity towards Rab11. The earlier paper by Westlake and colleagues showed through genetic and biochemical interaction assays that Evi5 binds Rab11a and Rab11b in a GTP-dependent manner, but exhibited no GAP activity towards Rab11 in in vitro assays [42]. Despite that, endogenous Evi5 co-localizes with Rab11 in a complex in vivo and overexpression of Rab11 redistributes centrosomal cytoplasmic-localized Evi5 to Rab11-positive recycling endosomes, in U2OS cells, thus suggesting a relationship between Rab11 and Evi5. Also, Rab11 effector proteins such as Rab11 family interacting protein 3 (Rab11-FIP3), which bind to active, GTP-bound Rab11, could compete with Evi5 for Rab11 binding. Another paper by Dabbeekeh et al. also found that during interphase in the HeLa cell model, Rab11 and Evi5 co-localized at the pericentriolar region. Furthermore, Evi5 was shown to interact with Rab11 in a GTP-enhanced manner via its TBC domain. Contrary to the previous report, these authors confirmed that Evi5 exhibited specific Rab GAP activity in vitro towards Rab11 (in comparison with similarly endosome-localized Rab4) [43].

4. Evi5's association with the Rab11 subfamily of Rab GTPases

The Rab11 subfamily (Rab11a, Rab11b and Rab25) and their effectors [44] mediate trafficking steps at and from the recycling endosome [45,46] of a large variety of molecules such as ligand transporters [47,48], ion channels [49–51] signal receptors [52–58], cell adhesion molecules [59,60] and others [61]. Endosomal recycling activities mediated by the Rab11 subfamily are key events in various physiological processes such as regulation of cell polarity [62], cell fate determination [63], tissue morphogenesis [64,65], primary ciliogenesis [66], autophagy [67], membrane trafficking and cytokinesis [15,16,68,69], and cell migration [70,71]. Disruption of Rab11-mediated traffic could underlie pathologies associated with neurodegenerative disorders such as Huntington's disease [72,73], while aberrant Rab11 family-mediated traffic have been associated with cancer cell migration and invasion [60,74].

Evi5's interaction with Rab11 suggests that it could potentially have a wide range of physiological and developmental roles, many of which still lack elucidation. Foremost, Evi5 may play an important role in the regulation of abscission via Rab11 and Rab11-FIP3, because silencing of Evi5 by RNA interference disrupted localization of Rab11-FIP3 complexes at abscission sites of all randomly selected cells undergoing final stages of cytokinesis [42]. During cell division, Rab11-FIP3 has been implicated in the membrane delivery from endosomal recycling compartments to sites of membrane

insertion (Fig. 3). Detailed analysis of the roles played by Evi5 during cytokinesis could potentially be complicated by its apparent involvement in the earlier stages of the cell cycle, as discussed in the previous section. The understanding Evi5 and Rab11's possible role in cell migration has recently been further deepened. An RNAi-based screen for Rab GAPs involved in collective cell migration of *Drosophila melanogaster* border cells (BCs), which has been known to be dependent on protein trafficking, identified the fly orthologue of Evi5 as a key regulator of the BC migration [75]. Evi5 acts through Rab11, functioning as a Rab GAP for Rab11. In BC migration towards the oocyte, polarized spatial localization of receptor tyrosine kinase (RTK) activity at the leading edge is required. Moreover, an earlier paper by the same group showed that Rab11 is essential in controlling the activation of RTKs at the leading edge of these cells [76]. Both overexpression and depletion of *Drosophila Evi5* abolished enrichment of phosphotyrosine signals at the front of the cell cluster, indicating that stringent regulation of Rab11 activity is necessary for the proper spatial restriction of activated signaling receptors [75]. Acting as a Rab GAP of the Rab11 family therefore, Evi5 plays clear and important roles in modulating cytokinesis and cell migration during development.

5. Evi5 in human diseases

The association of Evi5 with important cell cycle and cell migration regulators suggests its involvement in the pathology of certain diseases. Early work associated with Evi5's identification points towards its role as an oncogene or tumor suppressor [2,3]. This notion is strengthened by its interactions with Emi1 and Rab11, with an apparent role in the regulation of the cell cycle through interaction with the aurora-B kinase protein complex [4,12]. However, direct evidence for Evi5's role in oncogenesis, or suppression of tumorigenesis, is still lacking. Evi5 has been found to be overexpressed in certain cancer types [77,78]. For instance, Evi5 could serve to prevent exhaustion of hematopoietic stem cells, whose levels are elevated as a result of the loss of tumor suppressor Runt-related transcription factor 1 (RUNX1) [78]. This suggests that elevated Evi5 levels could promote leukemia in cooperation with a loss of RUNX1. It is also conceivable that loss of Evi5 may enhance cancer cell invasion and metastasis via an enhanced Rab11 family GTPase activity, but this possibility remains to be clearly demonstrated.

An unexpected Evi5 disease link has arose from recent genomic analyses, which implicated Evi5 as a risk factor for multiple sclerosis (MS), a rather common autoimmune demyelinating disease with multiple neurological symptoms [79]. MS has a modest degree of heritability, and the Human Leukocyte Antigen (HLA) region is a known strong susceptibility locus for MS [80]. Genome-wide association studies have since identified other susceptibility genes, including *CD25*, CD58, *CLEC16a*, Interleukin-2 Receptor α (*IL2RA*), Interleukin-7 Receptor (*IL7R*), G5 (*GPC5*), Regulator of G protein Signaling 1 (*RGS1*), and Tyrosine Kinase 2 (*TYK2*) [81–83], with some of these genes having clear functions in T-cell-mediated immunity.

Several separate analyses have associated single nucleotide polymorphisms (SNPs) of Evi5 with increased MS susceptibility [82,84–89]. The molecular basis of this association is unclear, as a role for Evi5 in T-cell mediated immunity has not yet been explored extensively. However, it is conceivable that Evi5 could affect aspects of innate immunity such as toll-like receptor signaling through Rab11, which was shown to be responsible for transport of the receptor via recycling endosomes [57]. A recent analysis of enhancer elements bound by the CCCTC-binding protein (CTCF) indicated that MS susceptibility SNPs within the last intron of

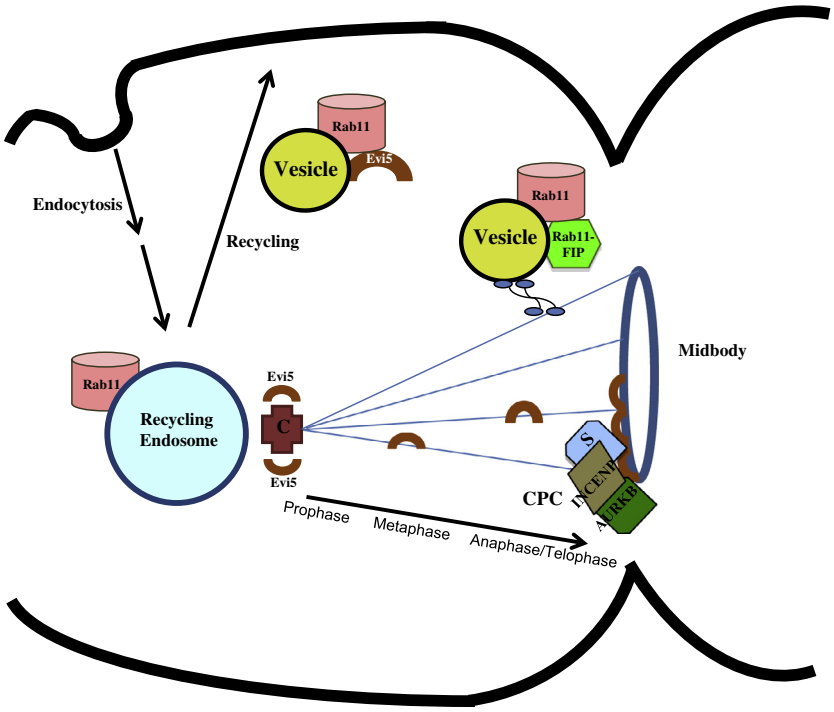

Fig. 3. Evi5's role in cytokinesis. Rab11a (Rab11) and Evi5 have both been localized to the pericentriolar region in U2OS cells (the cross indicated as 'C' in the diagram). Rab11a, together with its effectors (Rab11-FIP), are required to target recycling endosomes to the midbody or cleavage furrow, carrying physical membrane and factors important for cytokinesis. Evi5's presence at the interphase centrosomes and recycling endosomes could inhibit FIP's promotion of cytokinesis [42]. It was also noted that in prophase, Evi5 appears to have a cytoplasmic localization. However in metaphase, Evi5 become associated with the mitotic spindles and it moves to the spindle midzone and midbody at the final stages of mitosis. At these late stages Evi5 also interacts with the Chromosomal Passenger Complex (CPC) INCENP-Aurora kinase B (AURKB)-survivin (S) for the completion of cytokinesis [12]. From the different views on Evi5's localization, it is possible that Evi5 plays not one, but possibly several roles in cell division, with the differing roles depend critically on its spatial or temporal localization (that are in turn regulated by ubquitination and degradation).

the *EVI5* gene, likely CTCF-binding enhancer elements, affected the expression of the neighboring gene and not Evi5 itself [89]. That Gfi1 and not Evi5 being the causal gene for MS susceptibility, at least pertaining to some of the SNPs, makes intuitive sense, as Gfi1 is a known regulator of lymphocyte development and activation [90].

6. Functional roles of Evi5-like (Evi5L)

While Evi5 has been implicated in a variety of roles and several diseases, the function of Evi5L, a paralogue of Evi5, is more elusive. It bears approximately 70% homology to Evi5, and hence would be expected to interact to some degree with Evi5 interacting partners, yet, no interaction has been demonstrated. Unlike Evi5, Evi5L appears to be largely cytosolic throughout the cell cycle, but has an interesting apparent concentration at the pericentriolar region (Fig. 4). Evi5L was first shown to bind Rab10 and exhibits in vitro GAP activity towards the small GTPase [5]. Rab10 is important in the regulation of endocytic recycling, and has been implicated in multiple roles that include basolateral recycling of clathrin-independent cargo in *Caenorhabditis elegans* intestine [91], AMPA-type glutamate receptors in post-synaptic membranes [92], basolateral recycling in polarized Madin–Darby canine kidney (MDCK) cells, Glut4 transporter trafficking in response to insulin [93], Toll-like receptor 4 (TLR4) signaling [94], phagosome maturation [95], and ciliary transport [96]. Notably, except for primary ciliogenesis, the role of Evi5L in these processes has not yet been demonstrated.

The primary cilium is a singular cellular projection protruding from the surface of cells that concentrates various signaling receptors and molecules. Hence, it is considered to be a signaling nexus that plays key roles in cellular physiology and organism development [97,98]. In fact, defects in the primary cilium's functional and trafficking components underlie multiple human ciliopathies [99]. Screening for Rab GAPs that affect primary cilium formation, Barr and colleagues identified Rab8a, −17, and −23, and their cognate GAPs, XM_037557, TBC1D7, and EVI5L. Carried out in the human telomerase-immortalized retinal pigmented epithelial (hTERT-RPE1) cell model, cells overexpressing Evi5L significantly reduced primary cilium formation by more than half [100]. In the in vitro GAP activity assays, the authors showed that Evi5L especially exhibited specific activity towards Rab23, but not Rab10.

Rab23 was first isolated based on a mouse mutation to a gene whose functions antagonized Sonic hedgehog (Shh) signaling during embryonic development [101]. Shh signaling is critically dependent on the primary cilia, as proper functioning of ciliary intraflagellar transport (IFT) processes are essential for Shh signaling [102,103], and key components of the Shh signaling pathway, such as the Gli family of transcription factors and smoothened, are enriched at the primary cilia [104,105]. Although Rab23 has not yet been shown to be critical for ciliogenesis and ciliary transport as shown for Rab8a [106,107] and Rab11 [66], it is known to influence the levels of smoothened in the cilium [108]. Notably, in humans, Rab23 mutations underlie the pathological phenotypes associated with Carpenter's syndrome [109], whose phenotypes bear resemblance to those found in some ciliopathies, especially obesity, polydactyly and certain malformations of the brain [109]. Although the exact ciliary cargo involved and transport step mediated by Rab23 and Evi5L are yet unclear, accumulating evidence suggest that they have important roles in specific ciliary transport.

7. Future perspectives

We have summarized and discussed above cellular and physiological activities and roles of the Evi5 family of TBC proteins. The tumorigenic (or tumor suppressive) activity of Evi5 requires further clarification. Its ability to prevent hematopoietic stem cell exhaustion [78] suggests that Evi5 might have a more general role in adult stem cells. It is also not known if Evi5 has a direct role in cancer cell migration and invasion via its interaction with Rab11GTPases and the Rab coupling protein (RCP) [110]. Many potential functional aspects of Evi5 and Evi5L remain unexplored. One possibility pertains to a plausible role for Evi5 in ciliary transport and ciliogenesis via its GAP activity on Rab11 [66]. A recent report showed that Evi5 levels are dramatically elevated during blastema formation in salamander limb regeneration [111]. The

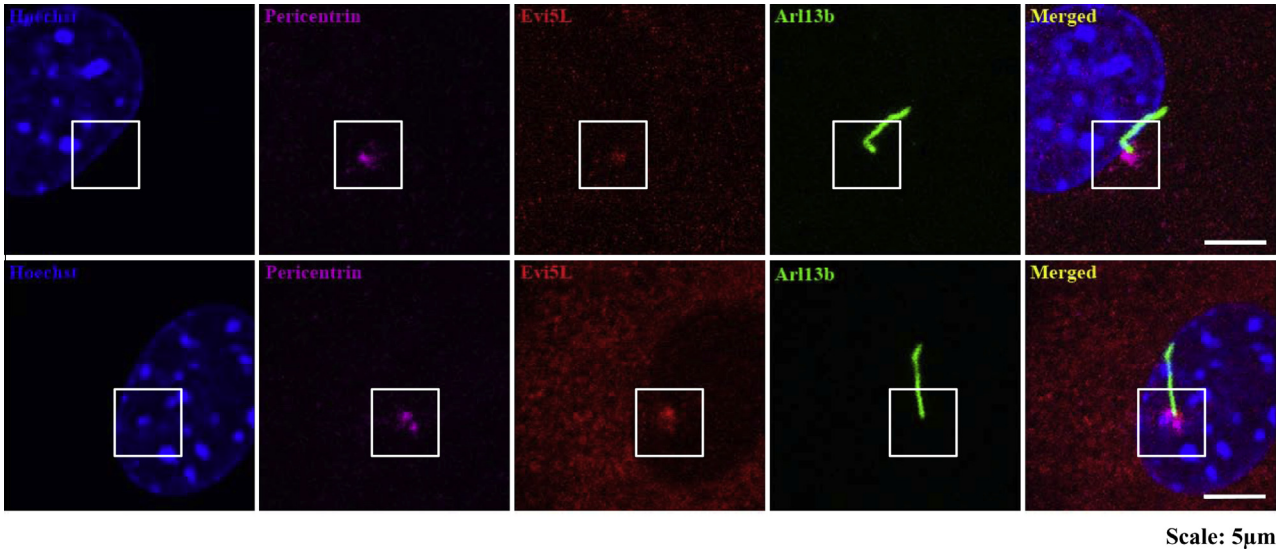

Fig. 4. Subcellular localization of Evi5L. Top panel: A magnified image of Evi5L in NIH3T3 mouse fibroblasts using an Evi5L antibody from Abcam (ab88915). Endogenous Evi5L (red) appears to have a generally diffused cytosolic staining in NIH3T3 mouse fibroblasts. Cells are transfected with Arl13b-GFP (Origene: MG206808) (green), a primary cilium marker, and also labeled with Pericentrin (Abcam; ab4448) (magenta). Bottom panel: A magnified image of an NIH3T3 mouse fibroblast that is transiently overexpressing both mouse Arl13b-GFP (green) and human Evi5L (Openbiosystems: MHS1011-76129), and co-stained with Pericentrin (Abcam; ab4448) (magenta) and Evi5L (ab88915) (red). In both panels of images, primary cilia were induced after treatment with 24 h of serum starvation media. From the panel of images, it appears that Evi5L staining exhibits a partial co-localization with the pericentrin staining. Scale bar = 5 μm.

authors postulated that high levels of Evi5 function to arrest dedifferentiated cells in the G1/S/G2 phases of the cell cycle until they have accumulated under the wound epidermis, with entry into mitosis subsequently triggered by growth factors. It would be interesting to see if Evi5 levels are also similarly elevated during regenerative attempts in mammalian tissues, which we know have a vastly inferior regenerative capacity compared to amphibians. Similarly, it would also be interesting to investigate Evi5's role in cell migration during wound healing.

While activity of Evi5 may be restricted by its nuclear and centrosomal localization [11], Evi5L is largely cytosolic, with some concentration around the pericentriolar region (Fig. 3). It could conceivably participate in trafficking events in multiple membrane compartments. While its overexpression clearly affects primary ciliogenesis [100], its exact mode of function is unclear. Although Evi5L exhibits specific Rab23 GAP activity in vitro, Evi5L's role in Shh signaling has not been reported. Moreover, whether it functions strictly through Rab23 is unclear. Alternatively, its suspected in vitro interaction with Rab10, reported by one group [5] but not another, is worth further exploration. Thus far, Evi5 and Evi5L appears to have non-overlapping Rab targets and functions, it would be interesting to see if these could, at least in some cases, complement the deficiency of each other when overexpressed.

Investigating the possible underlying cellular mechanisms of how Evi5 polymorphisms influence onset and progression of diseases such as MS would be of translational importance. Current animal models of MS mimic disease pathology, but not its genesis. It would be worth checking if transgenic expression of Evi5 and Gfi1, or their specific ectopic expression in the central nervous system in animal models will either produce MS pathologies, or enhance the susceptibility to MS induced by neurochemical means.

Acknowledgements

The authors declare no conflict of interest. B.L.T. is supported by the NUS Graduate School of Integrative Sciences and Engineering. We are grateful to the constructive comments from both reviewers, which improved the manuscript.

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